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Assessment of genetic diversity and population structure of swamp eel *Monopterus albus* in China

Hongwei Liang*, Shanshan Guo, Zhong Li, Xiangzhong Luo, Guiwei Zou**

Yangtze River Fisheries Research Institute, Chinese Academy of Fishery Sciences, Wuhan, 430223, Hubei province, China

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ABSTRACT

Swamp eel has become one of the most economically important fish in China. However, the wild swamp eel is facing the serious challenge of declining population and germplasm degeneration because most of farming swamp eel fingerlings was collected by fishing wild individuals. In this study, the genetic variation of *Monopterus albus* in six dominant farming regions was investigated based on the mitochondrial DNA D-Loop of 1008 bp in length. 180 individuals from 6 populations were examined and 74 haplotypes were observed. The overall genetic diversity was abundant and which its SD population was highest but CQ population was lowest. There was obvious genetic differentiation among investigated populations. Phylogenetic analysis revealed that these individuals were divided into four distinct genetic clades, clade A, B, C, and D. Clade A should be the most common ancestor clade. AH and CQ populations might originate from one single ancestor in maternal clade A. Clade C should be a native important clade in China. Though the genetic diversity did not suffered obvious decreasing, it is still imperative to take effective conservation measurements and establish an efficient selective breeding program.

1. Introduction

The swamp eel (*Monopterus albus*) is a family of freshwater eel-like teleost fishes, which belongs to the family Synbranchidae, order Synbrachiformes and class Actinopterygii. It originates widely in tropical, subtropical and temperate freshwater regions from Southeast Asia to East Asia (Zhou et al., 2002) and commonly found in rice fields, swamps, ponds, and muddy areas (Siang et al., 2007; Li et al., 2013). Because of its delicious taste, high nutrition and medicinal value, swamp eel has become one of the most economically important economical fishes, especially in China (Qu et al., 2014; Hu et al., 2015a). The worldwide production of *Monopterus albus* has reached 321,006 tons in 2012 (FAO Yearbook Fishery and Aquaculture Statistics, 2014), while most of the production was provided Chinese fisheries (320,966 tons, China Fisheries Yearbook, 2013).

Swamp eel is distributed throughout most regions, except the Qinhai-Tibet Plateau in China. In 2013, the total annual production of swamp eel was 346,077 tons (China Fisheries Yearbook, 2014). Six dominant farming regions, which are Hubei, Jiangxi, Anhui, Hunan, Sichuan, and Shandong, have the first six production with 161,837 tons, 79,471 tons, 43,643 tons, 29,999 tons, 11,409 tons, and 2083 tons, respectively (China Fisheries Yearbook, 2014). Nowadays most of the cultured swamp

* Corresponding author. Yangtze River Fisheries Research Institute, Chinese Academy of Fishery Sciences, No.8, 1st Wudayuan Road, Donghu Hi-Tech Development Zone, Wuhan, 430223, China.
** Corresponding author.
E-mail addresses: lianghw@yfi.ac.cn (H. Liang), zougw@yfi.ac.cn (G. Zou).

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eel fingerlings are collected from fishing wild larvae. Thus, the wild swamp eel resources are facing serious challenges with the populations declining due to overfishing and large-scale application of pesticides (Yang et al., 2011a; Lei et al., 2012). What's more, the germplasm quality has declined sharply in terms of resistance and growth (He et al., 2010; Shao et al., 2015). In order to conserve and utilize this species well, it is vital to carry out fundamental breeding research. In the past ten years, some researchers have started utilizing artificial reproduction (Bing, 2005), culture technology (Yang et al., 2011b; Ma et al., 2014), sex differentiation of the swamp eel (Zhou et al., 2002; Hu et al., 2014).

Genetic diversity provides the fundamental material for biological diversity and selective breeding (Frankham et al., 2002; Hao et al., 2006). The genetic structure investigation is an important step to gain background knowledge about all kinds of species. Some studies analyzed the genetic variation of swamp eel by using different molecular markers, such as RAPD (Yin et al., 2005), microsatellites (Li et al., 2013; Lei et al., 2012), ISSR (Li et al., 2013), and mitochondrial DNA (Cai et al., 2008). All of these studies involved a small sample or a limited distribution, such as the Sichuan Basin (Cai and Zhang, 2011; Cai et al., 2013), south China (Sun et al., 2015), and the Anhui province (Hu et al., 2015b). However, not all populations from the dominant farming districts of swamp eel were used to analyze and evaluate the genetic diversity. It will be very useful to compare the dominant cultured populations to establish basic breeding populations. To determine the genetic variations and investigate the genetic background of dominant farming districts, six populations are analyzed in this study.

2. Materials and methods

2.1. Sample collection and DNA extraction

The experimental procedures for swamp eel were performed according the standards of the Animal Care Policy of YFI (Yangtze River Fisheries Research Institute, Chinese Academy of Fishery Sciences). The alcohol-preserved muscle tissues obtained from 180 individuals of wild swamp eel were collected to six cultured dominant regions, including Hubei (HB), Jiangxi (JX), Anhui (AH), Hunan (HN), ChongQing (CQ, representing Sichuan Basin) and Shandong (SD). The detailed information of sample is listed in Table 1 and Fig. S1. Total genomic DNA was extracted by the described method (Taggart et al., 1992).

2.2. Mitochondrial DNA D-loop amplification and sequencing

The complete mitochondrial DNA D-Loop sequences of 180 samples were amplified using the primers 5′ TCAAATCCCTCTCATTACTCA 3′ and 5′ GATAAAGCCAGGACCAAAC 3′, which designed according the swamp eel mtDNA sequence deposited in GenBank (accession number KP779624.1). Each 30 μL PCR reaction system contained 2 μL of 10 mM each primer, 2U *Taq* DNA polymerase (TaKaRa, Japan), 3 μL 10 × PCR buffer (100 mM Tris-HCl, 500 mM KCl, 15 mM $MgCl_2$; TaKaRa), 2 μL 10 mM dNTP (TaKaRa, Japan) and about 100 ng DNA template. PCR amplification was carried on S1000™ Thermal Cycler (BIO-RAD, USA) using the following procedure: pre-denaturing at 95 °C for 3 min; 35 cycles of denaturing at 94 °C for 30 s, annealing at 58 °C for 30 s, and extending at 72 °C for 45 s; and a final extension at 72 °C for 5 min. The PCR products were detected by 2.0% agarose gel in 1 × TBE buffer at 80 V for 1 h and then purified using a DNA Agarose Gel Extraction Kit (Axygen, USA). The purified PCR products were cloned into pMD 18-T vector and sequenced by ABI 3730 automated sequencer (Applied Biosystems, USA).

2.3. Data analysis

All the sequenced D-loop fragments were edited and aligned using Clustal W program (Larkin et al., 2007). The genetic diversity parameters were analyzed by DNAsp5.0 program (Librado and Rozas, 2009), which included the number of polymorphic sites (S), number of haplotypes (h), haplotype diversity (Hd), nucleotide diversity (π), and average number of nucleotide differences (K). Genetic distance, fixation index, molecular variance, and neutrality tests were calculated by using Arlequin version 3.1 (Excoffier et al., 2005).

Based on the haplotypes of complete mitochondrial D-Loop sequences, the molecular phylogenetic tree of neighbor-joining (NJ) was constructed by MEGA version 5.0 (Tamura et al., 2011) and set the *Synbranchus marmoratus* (GenBank

Table 1
Samples collection information.

Population	Abbreviation	Location	Longtitude (E)/latitude(N)	Sample size
Hubei	HB	Qianjiang, Hubei Province	112°45′/30°06′	30
Jiangxi	JX	Xinyu, Jiangxi Province	114°56′/27°49′	30
Anhui	AH	Hefei, Anhui Province	117°16′/31°51′	30
Hunan	HN	Yueyang, Hunan Province	113°09′/29°37′	30
Shandong	SD	Weishanhu, Shandong Province	117°58′/34°53′	30
Chongqing	CQ	Chongqing	106°31′/29°32′	30

accession number AP004439.1) as the out-group. The Median-joining networks were generated by Network version 4.6.1 (http://www.fluxus-engineering.com/) (Bandelt et al., 1999) to which could analyze their relationship among haplotypes.

3. Results

3.1. D-loop sequence polymorphism

The complete mtDNA D-Loop sequences with 1008 bp in length were obtained from 180 wild individual samples. The average nucleotide composition was 32.1% A, 31.0% T, 23.7% C and 13.2% G, contents A + T (55.8%) was higher than that of C + G (44.3%), which showed A and T were richer in the swamp eel mtDNA D-Loop region. 212 polymorphic sites were found in the six populations. The HB population had the most polymorphic sites (N = 173) and the CQ population had the least (N = 4) in all the populations (Table 2). 123 of the 212 polymorphic sites were singleton variable sites and the other 89 were parsimony informative sites.

3.2. Population diversity

The genetic diversity of six populations was shown in Table 2. There were 74 haplotypes that were identified in the dominant farming districts. The lowest number of haplotypes was found in the CQ population (N = 2), while the highest number of haplotypes was observed in the SD population (N = 28). Among 74 haplotypes, 60 were unique haplotypes detected in the 180 individuals, and other haplotypes were shared by more than two populations. The haplotype 10 (Hap10) with the highest frequencies presented 27 times and was only observed in the CQ population. The diverged nucleotide diversity and haplotype diversity were exhibited in different populations. The nucleotide diversity values ranged from 0.001 to 0.017 and the overall value was 0.013 for six populations. The lowest nucleotide diversity was in the CQ population and the highest one was in the HB population. The JX and SD populations had the same nucleotide diversity of 0.015. The haplotype diversity values ranged from 0.186 to 0.995, which the lowest was in the CQ population and the highest was in SD population. The overall haplotype diversity in the six populations was 0.946. According the results, the CQ populations had the lowest nucleotide diversity and haplotype diversity, which had the lowest genetic diversity among the six populations.

3.3. Analysis of molecular variance (AMOVA)

Molecular variance (AMOVA) based on the mtDNA D-Loop sequences of six populations was analyzed. The results showed that the genetic variation was 66.09% among swamp eel individuals within populations, while genetic variation was 33.91% among their populations. These results indicated that most variation occurred within the population. The Fst (fixation index), involved in the population's genetic differentiation, was 0.339 among all the populations, which revealed that it had obvious genetic differentiation among the six populations.

3.4. Genetic distance among swamp eel populations

The genetic distance of the six populations ranged from 0.0089 to 0.0206 (Table 3). The furthest genetic distance was between the HN and HB populations, while the closest was between the AH and JX populations. The pairwise genetic distance within population ranged from 0.0008 to 0.0186, which the furthest was within the HB population and the closest was within CQ population. The highest fixation index was between the AH and CQ populations (0.8875) and the lowest range was between the SD and JX populations (0.0253). Among six populations, most of fixation indexes were more than 0.05 except one between the SD population and the HB population (0.0253).

The neutrality tests were conducted (Table 4). All Tajima's D values showed that there were not significantly different in populations except the HB population, which was −2.356 ($P < 0.01$). While Fu's Fs index of the AH and SD populations were −3.365 and −11.734, respectively, all shown significantly ($P < 0.01$).

Table 2
Genetic diversity results for six populations based on D-Loop sequences in this study.

Population	*h*	*S*	*Hd* (mean ± SD)	π (mean ± SD)	*K*
AH	9	9	0.708 ± 0.074	0.002 ± 0.000	1.506
CQ	2	4	0.186 ± 0.088	0.001 ± 0.000	0.745
HB	19	173	0.910 ± 0.045	0.017 ± 0.008	16.694
HN	8	40	0.591 ± 0.100	0.009 ± 0.003	8.703
JX	18	81	0.922 ± 0.001	0.015 ± 0.003	14.637
SD	28	85	0.995 ± 0.010	0.015 ± 0.003	15.529
Overall	74	212	0.946 ± 0.008	0.013 ± 0.002	13.311

Note: h: number of haplotypes; *Hd*: haplotype diversity; π: nucleotide diversity; *K*: average number of nucleotide differences.

Table 3
Pairwise genetic distance and fixation index between populations.

Population	AH	CQ	HB	HN	JX	SD
AH	**0.0015**	0.8875	0.3752	0.6447	0.1227	0.3403
CQ	0.0102	**0.0008**	0.2312	0.6594	0.4374	0.3471
HB	0.0162	0.0124	**0.0186**	0.3464	0.1461	0.0253
HN	0.0144	0.0140	0.0206	**0.0087**	0.3698	0.3436
JX	0.0089	0.0137	0.0192	0.0182	**0.0140**	0.0746
SD	0.0124	0.0120	0.0170	0.0178	0.0155	**0.0146**

Note: Pairwise genetic distance within population is at diagonal; genetic distance is below diagonal; fixation index is above diagonal. * Significant at $\alpha = 0.05$ after Bonferroni correction.

Table 4
Neutrality tests of 6 wild swamp eel populations.

Populations	Tajima's D	Tajima's D P-value	Fu's Fs	Fu's Fs P-value
AH	−1.048	0.138	−3.365*	0.024
CQ	−0.674	0.284	2.540	0.869
HB	−2.356*	0.000	0.623	0.630
HN	−0.508	0.307	5.414	0.967
JX	−1.102	0.121	−0.315	0.475
SD	−1.051	0.144	−11.734*	0.001

Note: Asterisks show significant differences at $P < 0.05$.

3.5. Phylogenetic analysis

A neighbor-joining tree was constructed based on 74 haplotypes from complete mtDNA D-Loop sequences of swamp eel, meanwhile the *Synbranchus marmoratus* (GenBank AP004439.1) was used as an outgroup (Fig. 1). The reliability of NJ tree topology was calculated with 1000 bootstrap replications. The result showed that 74 haplotypes belonging to 180 individuals were divided into four distinct genetic clades, clade A, clade B, clade C, and clade D. Of all these clades, Clade A consisted of 67 haplotypes from 160 individuals. Clade B, Clade C, and Clade D had two haplotypes, four haplotypes, and one haplotype, respectively.

Clade A was dominant in the four clades because it had the most haplotypes. Hap10 and Hap11, existing only in the CQ population, were clustered into clade A. The HN population had eight haplotypes (Hap30, Hap31, Hap32, Hap33, Hap34, Hap35, Hap36, and Hap37), seven of them were clustered to clade A and another one (Hap32) was sorted into clade B. Clade A included all individuals (60/60) of the AH and CQ populations, 93.3% of the individuals (28/30) from the HB population and 86.7% of the individuals (26/30) of HN, JX, and SD populations. The results showed the geographical distribution of different mitochondrial clades in Chinese swamp eel.

The result of Median-joining network (Fig. S2) based on the 74 haplotypes constructed by the NETWORK program showed that all the haplotypes were divided into four clades. Clade A had the most haplotypes and only one haplotype was in clade D. All haplotypes of the CQ population were sorted exclusively into clade A. The network results showed the similarity to the neighbor-joining tree.

4. Discussion

Mitochondrial DNA was widely used to analyze the population genetic structure and phylogeny in different species. The mitochondrial D-Loop region, with the highest variation and mutation rate of mtDNA, has been thought as an appropriate marker to study the genetic diversity and genetic differentiation in fishes, such as common carps (Thai et al., 2005; Liang et al., 2009) and tilapia (Szitenberg et al., 2012; Zou et al., 2015). The aquaculture of swamp eel as an economically important fish has made rapid progress, however the genetic resources of wild swamp eel has suffered serious challenge because of overfishing (Lei et al., 2012). Thus, background knowledge of genetic diversity and population structure is necessary to effectively protect and sustainably utilize swamp eel.

In this study, the genetic diversity of six wild populations from dominant culturing regions in China was analyzed. The results showed overall haplotype diversity was high in the six investigated populations. Among them, genetic diversity from CQ population of *Monopterus albus* was the lowest. Only two haplotypes with four variation sites were observed and uniquely existed in the CQ population. Cai et al. (2008) also analyzed the genetic diversity of swamp eel of Sichuan Basin and found that genetic diversity in Chongqing populations was low (Hd = 0.404, $\pi = 0.0007$). Lacking of genetic variation of swamp eel populations may result from the founder effects, intrinsic traits and/or genetic drift (Ray et al., 2015). The haplotype diversity (*Hd*) and nucleotide diversity is always used to measure the degree of polymorphism within a population. The haplotype diversity results showed that the genetic variation of swamp eel in the Anhui province were abundant (0.708). This result showed similar status to a previous study of swamp eel in the Anhui province based on *Cytb* (Hu et al., 2015a), partial D-loop

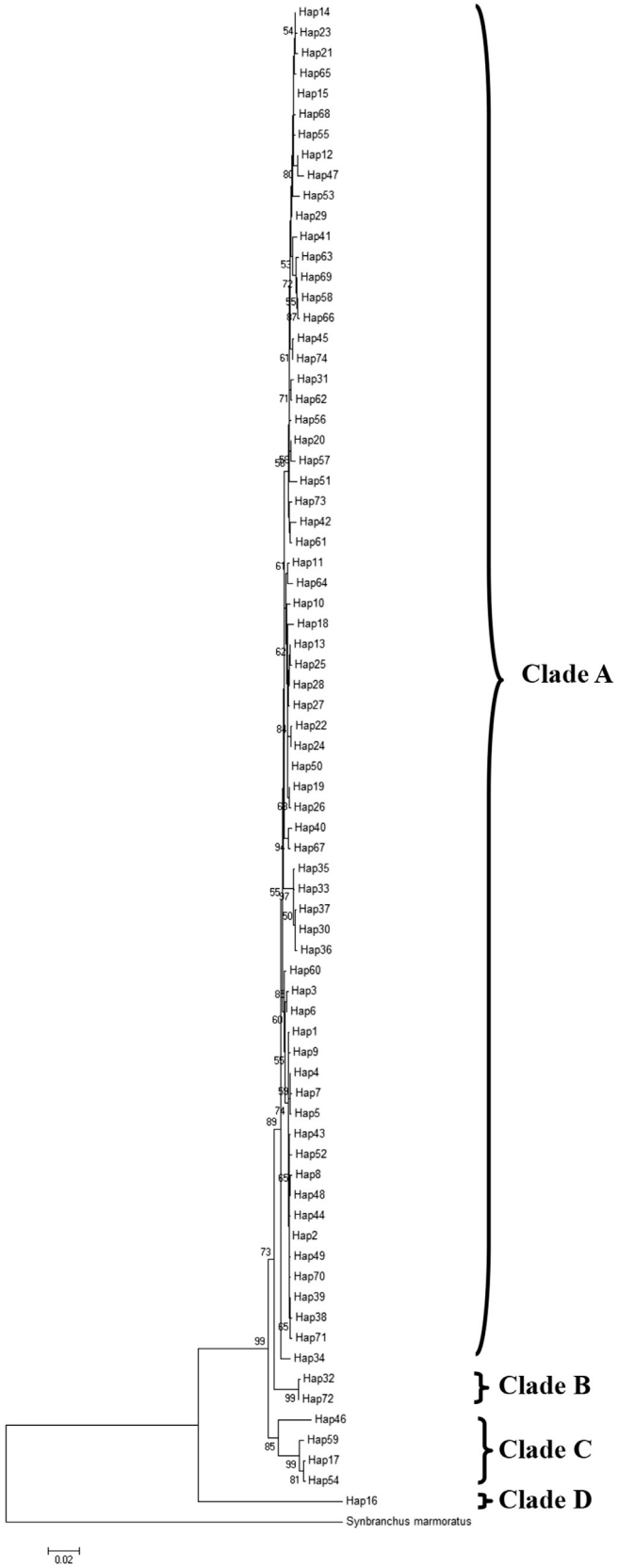

Fig. 1. The NJ tree based on 74 D-loop haplotypes of six swamp eel populations. The tree was constructed on the basis of Kimura two-parameter distance. *Synbranchus marmoratus* was the outgroup. Numbers on the nodes represent the bootstrap values for 1000 replications and only values greater than 50% are shown.

sequence (Hu et al., 2015b), and microsatellite analysis (Zhou et al., 2011). Except for the special population of the AH and CQ populations, the HB, HN, JX, and SD population displayed an abundant genetic diversity (Table 2). The haplotype and nucleotide diversity from Yueyang (Hunan province) were higher than the results of Sun et al. (2015). The HB population also showed more abundant genetic diversity (Hd = 0.910) than previous study results, which had been reported based on SSR (Zhou et al., 2011) and ISSR marker (Li et al., 2013). These results were caused by different research regions of mtDNA sequence and methods.

In previous reports, some studies focused on AH, HB, HN and Sichuan Basin and genetic diversity of their populations was AH > HB > HN > CQ (Zhou et al., 2011). However, genetic background of SD and JX population was scarce. In this study, the overall genetic variation of the six populations is SD > JX > HB > AH > HN > CQ. The level of genetic variation resulted from quantity of resource, natural environment, and other conditions (for example, aquaculture technique). Therefore, the number of wild swamp eel was decreasing rapidly and the population was suffering from a mix of different varieties and degeneration of good traits (Hu et al., 2015a; Shao et al., 2015). It was proven that the overall genetic diversity was abundant in the six dominant farming districts of swamp eel.

Swamp eel has the morphology characteristics such as a tapering tail and blunt snout, lacks pectoral and pelvic fins, as well as having rudimentary dorsal, anal, and caudal fins, with the caudal fin often absent. So it is difficult to study the taxonomy and phylogeny by recognizing the different morphology (Nico et al., 2011; Cai et al., 2013). Molecular phylogenetic and analytical techniques were the crucial methods to identify genetic divergence of swamp eel. Matsumoto et al. (2010) divided specimens from Southeast and East Asia into three clades based on geographical populations using mtDNA sequence. In this study, six populations were categorized into four highly divergent clades referred as clade A, B, C, and D (Fig. 1). Clade A was widely distributed in the six wild populations and had a dominant advantage in each population. It could be speculated that clade A was the most universal phylogenic clade and the common ancestor clade in evolution history of swamp eel. All individuals of the CQ population were sorted into clade A. This implied that swamp eel from the CQ population might be isolated to a single ancestor of maternal clade (Cai et al., 2008). In fact, some reasons can explain this single lineage of the CQ population. Firstly, because of the limited swimming ability, the swamp eel only lives in regular rice fields, ponds, rivers, and ditches of mud-holes. This reduced the chance for contact with other swamp eel populations. Secondly, the Chongqing municipality is located in the Sichuan Basin. As one of China's four largest basins, Sichuan Basin is surrounded by great mountains, which result in little impaction from external swamp eel populations. Thirdly, the swamp eel has the particular characteristic of mating among their population. To some extents, this controls the single genetic origin mate in the population and has little chance to other populations. On the other hand, the AH population with 9 haplotypes which no haplotype shared with CQ population, was also sorted into clade A. It implied that the AH population also originated from one single clade A. Clade B included Hap32 and Hap72, which belong to the HN and SD populations, respectively. Clade C was shared by three populations (HB, JX, SD), which should be a native important lineage in China. Meanwhile, the clade D only consisted of one haplotype (Hap16) coming from the HB population.

In summary, a detailed genetic diversity of six wild populations of swamp eel was obtained from the dominant culture districts in China. The results showed the genetic diversity of SD population was the highest and that of CQ population was the lowest. AH originated from one single maternal clade A as CQ population. At present the genetic diversity was abundant and did not suffered obvious decreasing in the investigated swamp eel populations. In order to sustainably utilize the germplasm resources of swamp eel, further strategies should be taken to protect the current wild resources and accelerate the research of artificial reproduction and develop selective breeding programs for using in the production.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bse.2016.06.006.

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